WHITE MATTER CORRELATES OF NEUROPSYCHOLOGICAL FUNCTION IN YOUNG ADULT METHAMPHETAMINE USERS

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This manuscript is dedicated to my brother, Robin, who taught me much as he succumbed to his battle with addiction.

ABSTRACT

Background

Methamphetamine (MA) abuse is a global health concern due to widespread use and harmful effects, which includes neurotoxicity. This study aimed to describe neurocognitive deficits associated with MA dependence in young adults and to explore whether these deficits correlate with white matter (WM) microstructural abnormalities using diffusion tensor imaging (DTI).

Methods

Twenty-one MA dependent individuals recently enrolled in an outpatient rehabilitation program and nineteen healthy controls participated in the study. Each participant completed a neuropsychological evaluation and underwent diffusion tensor imaging within one week of testing. Average whole-brain fractional anisotropy (FA) and mean diffusion (MD) measures derived from DTI data were compared between groups. Group differences in performance within specific neurocognitive domains and in a composite global neurocognitive score (GNS) were tested using non-parametric univariate statistics and within a linear regression framework, adjusting for age and gender. Correlation analyses were conducted to test associations between the neuropsychological data and selected frontal white matter (WM) tracts, including the genu and body of the corpus callosum (CC); right and left cingulum bundle (CB); right and left uncinate fasciculus (UF); right and left anterior corona radiata (CR) and the right and left superior longitudinal fasciculus (SLF).

Results

No significant between-group differences were detected for performance in any of the neuropsychological domains assessed. No relationship between FA or MD and the GNS was demonstrated in the tracts of interest. After adjusting for age and gender, significant group differences in FA and MD were detected across several regions of interest (ROI), however, these did not survive corrections for multiple comparisons.

Conclusion

Cognitive performance and white matter integrity did not differ between young MA dependent subjects and healthy controls. Whatever differences that were found in white matter did not survive correction for multiple comparisons. These findings may reflect one or more of several possibilities: that brain function and structure is relatively preserved in younger individuals; or that differences were too small to be detected in this sample. Further studies should explore the effects of aging, poly-substance abuse and HIV co-infection on neurocognitive functioning and structural brain integrity in methamphetamine users.

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ABBREVIATIONS

(CUBIC) Cape Universities Brain Imaging Centre

(CB)	Cingulum Bundle
(CC)	Corpus Callosum
(CR)	Corona Radiata
(DAT)	Dopamine transporter
(DTI)	Diffusion tensor imaging
(FA)	Fractional anisotropy
(FSL)	FMRIB Software Library
(GNS)	Global Neurocognitive Score
(HREC)	Human Research Ethics Committee
(MA)	Methamphetamine
(MA +)	Methamphetamine user
(MA -)	Healthy control subject
(MD)	Mean diffusion
(MRI)	Magnetic resonance imaging
(PET)	Positron emission tomography
(ROI)	Region of interest
(SA)	South Africa
(SACENDU)	South African Community Epidemiology Network on Drug Use
(SLF)	Superior Longitudinal Fasciculus
(5-HT)	Serotonin
(TBSS)	Tract-based spatial statistics
(UCT)	University of Cape Town
(UDS)	Urine Drug Screen
(UF)	Uncinate Fasciculus
(WC)	Western Cape
(WM)	White matter

CHAPTER 1: INTRODUCTION

1.1 Context

1.1.1 Methamphetamine use in South Africa

Methamphetamine (MA), like cocaine, is a powerful stimulant drug of abuse with widespread illegal use. It's popularity peaked in the United States (US) in the 1960s and later in the 1990s, with production largely driven by small-scale informal laboratories making use of readily available ingredients, e.g. pseudo ephedrine in cold and flu medication (Ciccarone 2011). MA abuse is traditionally associated with certain subgroups (e.g. gang members; sex workers; truck drivers; gay men), although growing concern has been expressed over its escalating use in South Africa (SA), particularly the Western Cape population (Brecht et al. 2004; Pluddemann et al. 2010). Despite numerous negative consequences affecting social, physical, psychological and occupational well-being, South Africans continue to abuse MA. Furthermore, in the context of South Africa's high HIV prevalence, the associated risk of HIV exposure, increased transmission rates, treatment non-adherence and disease progression in individuals who abuse MA is of grave concern (Kapp 2009; Colfax et al. 2010; Carrico et al. 2011). This chapter will outline some of the known deleterious effects of MA on human brain structure and function and will further describe the ethical considerations necessary for research in this study population.

1.1.2 Methamphetamine and neurotoxicity

MA is a highly lipophilic, neurotoxic molecule capable of producing significant disruption in brain structure and function. Animal and human studies support a dose-related association between acute and chronic MA use and neurotoxicity. Rats exposed to MA exhibit decreased levels of striatal dopamine as well as destruction of dopaminergic nerve terminals (Ricaurte et al. 1982). Serotonergic function is also compromised following MA exposure as evidenced by: loss of the 5-HT transporter; 5-HT depletion; depletion of 5-

HT metabolites and reductions in tryptophan hydroxylase (as reviewed in Meredith et al 2005) (Meredith et al. 2005). Post mortem findings further support alterations in dopaminergic functioning seen in neuroimaging studies as evidenced by deficits in available dopamine, the dopamine transporter and tyrosine hydroxylase, a dopamine precursor (Wilson et al. 1996).

Proposed mechanisms of neurotoxicity include accumulation of reactive oxygen species and severe oxidative stress, glutamate mediated excitotoxicity and mitochondrial dysfunction, preferentially affecting striatal dopaminergic nerve terminals (Quinton & Yamamoto 2006). Davidson et al. further postulate that MA induced neurotoxicity occurs via two pathways i.e. loss of dopaminergic function in acute overdose (4 x 5mg/kg) and MA induced apoptosis seen in chronic use (15mg/kg/day x 14 days) (Davidson et al. 2001).

1.1.3 Methamphetamine and cognition

Long-term/chronic MA use has been shown to result in significant cognitive impairment affecting multiple domains. Up to 40% of long-term MA dependent individuals exhibit global neuropsychological impairment (Rippeth et al. 2004). Cognitive processes dependent on frontal-striatal and limbic circuits are predominantly affected in MA abuse, irrespective of age and level of education (Scott et al. 2007). The following domains have been shown to be impaired: episodic memory; prospective memory; executive function, complex information processing speed and psychomotor function (Scott et al. 2007; Rendell et al. 2008). Smaller effect sizes were noted in attention/working memory, language and visual construction. Successful rehabilitation, including therapeutic strategies, abstinence and impulse control, rely heavily on the aforementioned neuropsychological domains being intact. Impairment in areas such as learning, memory and impulse control may help to explain the difficulty experienced by MA users, engaged in a rehabilitation program, to refrain from using the drug (Gowin et al. 2013).

Despite increasing numbers of individuals using MA, few studies of this nature exist in South African populations. This research may further enhance our understanding of the complex maladaptive behaviours seen in substance-dependent individuals.

1.1.4 Methamphetamine and diffusion tensor imaging

Magnetic resonance imaging (MRI) plays an increasingly important role in understanding brain structure and function in both normal and diseased states. Diffusion tensor imaging (DTI) was introduced in 1994 and is one of several MRI modalities that allow for the non-invasive study of white matter integrity based on the free diffusion of water molecules within white matter tracts (Assaf & Pasternak 2007; Le Bihan et al. 2001). As reviewed in Salo and Fassbender, a rich body of literature of animal studies exists supporting the deleterious effects of MA on brain white matter, however, few human studies have been published (Tabled below) (Salo & Fassbender 2011). Furthermore, the combination of DTI parameters correlated with cognitive measures could provide a useful marker of human behaviour and response, for example, predicting treatment outcomes in a rehabilitation setting.

DTI employs the principle of Brownian motion i.e. the random movement of water molecules within a space. This motion is deemed isotropic if movement occurs equally in all directions. If water molecules are restricted in a plane e.g. along the axis of a neuron, movement will occur at different speeds with those along the axon moving the fastest compared to molecules moving perpendicular to the axis. This property of molecules in motion is called anisotropy and, with the aid of sophisticated MRI techniques, can be used to demonstrate the direction of water molecules along white matter tracts. These in turn allow clinicians to visualize anisotropic diffusion of water molecules within several cubic mm of white matter, known as a voxel, which may be representative of several disease states (Le Bihan et al. 2001). The resultant anisotropy allows for information to be gained on both tissue microstructure and architecture within the voxel. DTI has been successfully employed to

demonstrate disruption of brain tissue in a number of pathological states. Examples include: demyelination in multiple sclerosis and oedema in response to inflammation seen in acute cerebrovascular accidents (CVA) (Filippi et al. 2001; Sotak 2002).

Fractional anisotropy (FA) is the most frequently used parameter of DTI. FA provides information on the orientation and integrity of white matter fibers within a voxel. Each voxel consists of thousands of white matter fibers and a supportive matrix of glial cells, including oligodendrocytes responsible for the myelin sheath around the axon. FA comprises of an intensity scale that ranges between zero and one. FA is high (close to one) if diffusivity is rapid along fibres and approaches zero if diffusion occurs in all directions (Assaf & Pasternak 2007). Mean diffusivity (MD) provides an overall measure of diffusivity within a voxel or region. MD is understood to increase in pathological disease states e.g. inflammation and oedema (Le Bihan et al. 2001). FA is thought to decrease in the presences of tissue damage, due to either increased radial (RD) or decreased axial diffusivity (AD). More recently, imaging specialists have started to examine AD and RD as separate entities, in combination with FA, in order to glean more information on tissue structure (Alexander et al. 2007). Several limitations should be taken into consideration when interpreting data obtained using DTI. These include: artifact; thermal and physiological image noise; dilution of volume when averaged in larger voxels and fibre crossing (Alexander et al. 2007).

1.1.5 Literature review of DTI studies in methamphetamine dependence

A literature review conducted, using Pubmed Central, of DTI studies undertaken in MA dependence yielded the results summarized in Table X below. Keywords used in the search include: ‘DTI’, ‘diffusion tensor imaging’ ‘methamphetamine’, ‘MA’, ‘amphetamine’ and ‘structural imaging’ (Alicata et al. 2009; Chung et al. 2007; Ersche et al. 2012; Kim et al. 2009; Salo et al. 2009; Tobias et al. 2010; Lederer et al. 2015).

Table I: Literature review of DTI studies in MA dependence

Primary author	Year	Sample	Whole brain voxel analysis or ROI	Measure	Significant findings
Alicata	2009	30 MA +, 30 MA -; age 33.3 (8.6)	Frontal WM; parietal WM; genu CC; caudate; putamen; thalamus; cerebellar vermis	FA, ADC	MA +: decreased FA in right frontal WM; increased ADC in left caudate and bilateral putamen. Higher ADC in left putamen and higher right putamen was associated with higher MA doses.
Chung	2006	32 MA +, 30 healthy controls; men (n=23), women (n-9); age men 36.0 (6.7), women 29.0 (7.2)	Bilateral frontal WM; bilateral parietal WM; bilateral occipital WM. (frontal matter measured relative to the AC-PC plane	FA	MA +: Decreased FA in bilateral frontal WM at the AC-PC plane and right frontal WM above this plane. Less prominent in female participants.
Ersche	2012	50 sibling pairs with at least 1 sibling MA+, 50 unmatched controls	Tract-based skeleton	FA	Decreased mean FA in MA + and their siblings compared to healthy controls. Therefore observed WM abnormalities were shared among family members and may have pre-disposed them to drug-taking.
Kim	2008	11 MA + abstinent, 13 healthy controls (Not tested for HIV); age 34.4 (2.9)	Genu and splenium of the CC	FA	Decreased FA in genu; trend to decreased FA in splenium although not significant.
Lederer	2016	40 MA +, 40 matched controls	Genu CC, Cingulum, Uncinate fasciculus	FA, MD, parallel diffusivity, perpendic-ular diffusivity	Increased MD and increased perpendicular diffusivity in the genu of the corpus callosum. Increased MD in the right cingulum. None of the WM changes were significantly associated with aggression.

Salo	2009	37 MA +, 17 healthy controls	Genu and splenium of the CC	FA, ADC	No significant difference in FA and ADC across groups. Trend towards decreased FA in genu but not significant (p=0.9)
Tobias	2010	23 MA +, 18 healthy controls	1) Prefrontal WM: four levels inferior-superior to the AC-PC plane (as per Chung, 2007) 2) Midline genu; midline splenium; bilateral anterior, midcaudal and caudal superior CR; anterior and posterior limbs and genu of the internal capsule; and the perforant path of the hippocampal formation.	FA	Lower FA: Right prefrontal WM above the AC-PC; midline genu CC; left and right midcaudal superior corona radiate and right perforant fibres.

WM = White matter; AC-PC = Anterior commissure-posterior commissure
CR = Corona radiata

Few studies appear to have been conducted in this area despite many authors emphasizing the value of DTI in understanding microstructural abnormalities associated with MA addiction. Replication of these studies in a South African, MA dependent population, correlating neuropsychological deficits with white matter deficits seen on DTI, will be of great value and may provide further insight into understanding maladaptive behavioural patterns associated with MA abuse.

1.2 Ethical considerations

Good clinical practice principles ensure that research conducted in clinical populations adheres to sound ethical and scientific standards, at all times, in order to protect the rights, safety and well being of study participants. Several special considerations need to occur when research is conducted with human subjects who abuse substances. Firstly, ethical approval of the study protocol, in accordance with the principles laid down by the Declaration of Helsinki, must occur prior to the commencement of research. This research study was approved by the Human Research Ethics Committee (HREC) of the University of Cape Town on the 6th of July 2010 (HREC 263/2010), initially as part of a larger parent study, i.e. Methamphetamine induced deficits in neuro-and-social cognition in the context of HIV infection (Appendix A), and later as a separate protocol for the achievement of a Masters of Philosophy (MPhil) in Neuropsychiatry on the 29th of August 2014 [HREC 635/2014] (Appendix B). For the purpose of fulfillment of the MPhil requirements, this thesis focused exclusively on HIV negative, methamphetamine dependent individuals and HIV negative, healthy controls. Permission was obtained from both the City of Cape Town and the Provincial Government of the Western Cape to conduct research in the relevant treatment facilities (Appendix E).

According to the Belmont Report, the following ethical principles should guide all clinical research: respect for persons; beneficence and justice. I will address each principle individually with regards to this research study.
(i) Respect for persons: every participant was comprehensively informed of the study prior to enrollment. I presented an information session to both staff and incumbents at Matrix clinic on the known effects of MA on the brain to ensure adequate knowledge on the purpose of the research (Appendix C). Participation was voluntary and all subjects were informed that they were able to withdraw from the study at any point without negative consequences. All participants were actively enrolled in their rehabilitation programme and had full capacity, thus ensuring competence to sign informed consent. Acutely intoxicated participants were not considered for the study. The investigators covered all transport costs and participants were compensated with food vouchers for the two days spent engaged in study procedure. Participants were not compensated with cash at any point due to the risk of this being

used to purchase illicit drugs. Participants were free of undue influence and not coerced to participate in the study. (ii) Beneficence: the investigators maintained ongoing awareness of minimizing risk for participants while maximizing potential benefit without permanent injury at all times. Due to the cross-sectional nature of the study, potential risks mostly pertained to procedure on the study day. Study participants were informed of all study procedures i.e. neuromedical exam; neuropsychological testing and MRI, with their comfort and safety prioritized at all times. (iii) Justice: selection of study participants ensured fair distribution of the risks and benefits of the research. Members of the healthy control group comprised of family and friends of the MA dependent group and were invested in advancing scientific knowledge of MA abuse despite not being addicts themselves.

In addition to the above considerations, the counselors at Matrix were informed of those participating in this research study. With the participants consent, essential clinical information was shared with the counselor to ensure adequate support for the participant throughout the study process.

1.3 Author Guidelines for PSYCHIATRY RESEARCH: NEUROIMAGING

The journal Psychiatry Research is the official publication of The International Society for Neuroimaging in Psychiatry. The Neuroimaging section caters for a variety of publications related to brain imaging of psychiatric disorders including: structural imaging; effects of behavioural tasks and impact of medication within this realm. The journal carries an impact factor of 2.831 (© Thomas Reuters Journal Citation Reports 2014).

The selection of this journal is an appropriate platform for my study as it encompasses brain imaging of a known noxious agent to the brain (methamphetamine) and correlates these findings with cognitive deficits present in this study population. These cognitive deficits in turn are associated with certain deleterious behaviours e.g. executive dysfunction resulting in poor

planning and execution of rehabilitation endeavours. The findings of such a study may help to link structural imaging with associated human behaviours, furthering our scientific understanding of addiction and subsequent treatment planning. This journal is listed on the Thomas Reuters/Institute for Scientific Information (ISI) Web of Science list January 2014: ISSN 0925-4927; E-ISSN 1872-7506; Netherlands.

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CHAPTER 2: PUBLICATION-READY MANUSCRIPT

WHITE MATTER CORRELATES OF NEUROPSYCHOLOGICAL FUNCTION IN ABSTINENT, YOUNG ADULT METHAMPHETAMINE USERS

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Keywords: methamphetamine; diffusion tensor imaging (DTI); fractional anisotropy; mean diffusion; white matter; neuropsychological function

Abstract

Background

Methamphetamine (MA) abuse is a global health concern due to widespread use and harmful effects, which includes neurotoxicity. This study aimed to describe neurocognitive deficits associated with MA dependence in young adults and to explore whether these deficits correlate with white matter (WM) microstructural abnormalities using diffusion tensor imaging (DTI).

Methods

Twenty-one MA dependent individuals recently enrolled in an outpatient rehabilitation program and nineteen healthy controls participated in the study. Each participant completed a neuropsychological evaluation and underwent diffusion tensor imaging within one week of testing. Average whole-brain fractional anisotropy (FA) and mean diffusion (MD) measures derived from DTI data were compared between groups. Group differences in performance within specific neurocognitive domains and in a composite global neurocognitive score (GNS) were tested using non-parametric univariate statistics and within a linear regression framework, adjusting for age and gender. Correlation analyses were conducted to test associations between the neuropsychological data and selected frontal white matter (WM) tracts, including the genu and body of the corpus callosum (CC); right and left cingulum bundle (CB); right and left uncinate fasciculus (UF); right and left anterior corona radiata (CR) and the right and left superior longitudinal fasciculus (SLF).

Results

No significant between-group differences were detected for performance in any of the neuropsychological domains assessed. No relationship between FA or MD and the GNS was demonstrated in the tracts of interest. After adjusting for age and gender, significant group differences in FA and MD were

detected across several regions of interest (ROI), however, these did not survive corrections for multiple comparisons.

Conclusion

Cognitive performance and white matter integrity did not differ between young MA dependent subjects and healthy controls. Whatever differences were found in white matter did not survive correction for multiple comparisons. These findings may reflect one or more of several possibilities: that brain function and structure is relatively preserved in younger individuals; or that differences were too small to be detected in this sample. Further studies should explore the effects of aging, poly-substance abuse and HIV co-infection on neurocognitive functioning and structural brain integrity in methamphetamine users.

1. Introduction

Methamphetamine (MA) is stimulant drug of abuse known to have detrimental effects on brain structure and function (Courtney & Ray 2014). MA presents a global health concern due to its addictive nature, widespread use and neurotoxic properties. Informal methamphetamine labs/kitchens are able to produce cheaper, more potent MA and continue to drive this problem (Maxwell & Brecht 2011; Brecht et al. 2004). South Africa, particularly the Western Cape province, is facing a growing MA epidemic (Plüddemann et al. 2013). According to the South African Community Epidemiology Network on Drug Use (SACENDU), 35% of individuals seeking treatment for drug use in 2006 report MA as their primary drug of choice (Pluddemann et al. 2008).

This highly lipophilic molecule dramatically enhances the release of catecholamines (dopamine; noradrenaline and serotonin) in the central nervous system and partially blocks their re-uptake. The increase in available catecholamines further stimulates the corresponding postsynaptic receptors (Cruickshank & Dyer 2009). Disruption of these neurotransmitter systems is thought to underlie the predominantly frontostriatal pattern of neurocognitive dysfunction previously reported in MA dependent individuals (Sekine et al.

2003). Deficits in episodic memory; executive function; motor skills; information processing speed and language have all been described in acute and chronic MA use (Maxwell & Brecht 2011; Scott et al. 2007). Some of these data suggest that decision-making may be impaired in MA dependent individuals, leading to high-risk behaviours and poor treatment outcomes (Pluddemann et al. 2008; Paulus et al. 2002). Intact neurocognitive function is important to treatment planning (many of which are cognitively-based interventions) and long-term rehabilitation.

This study aimed to investigate whether a young adult, MA dependent, South African population display neurocognitive deficits, and associated WM abnormalities detected on diffusion tensor imaging (DTI). DTI is a magnetic resonance imaging (MRI) modality, which allows for the non-invasive, in-vivo study of brain white matter (WM) structure based on the free diffusion of water molecules within WM tracts (Assaf & Pasternak 2007). The extent to which water molecules move within the same orientation within white matter fiber tracts, known as anisotropic diffusion, can be used to infer tissue microstructural abnormalities in certain disease states i.e. demyelination; inflammation; oedema; neoplasia and stroke (Alexander et al. 2007; Le Bihan et al. 2001).

Fractional anisotropy (FA) is a parameter of DTI and provides information about the orientation of white matter fibers within a voxel. FA is high (close to 1) if diffusivity is rapid and uninterrupted along fibers whereas FA approaches zero if diffusion occurs in all directions (Assaf & Pasternak 2007). Mean diffusivity (MD) provides an overall measure of diffusivity within a voxel or region and is understood to increase in pathological disease states (Le Bihan et al. 2001). For instance, in the MA DTI literature, Chung and colleagues demonstrated disruption of frontal structures in abstinent MA users in the anterior-posterior commissure (AC-PC) plane, which correlated with an impaired performance on the Wisconsin Card Sorting Test (WCST), a measure of executive functioning (Chung et al. 2007). Their group reported that male MA dependent subjects had significantly lower FA values in frontal white matter, compared to female MA participants and more errors on the

WCST. Kim et al. (2009) further demonstrated abnormal WM in the genu of the corpus callosum (CC) with corresponding impairment on the WCST. Lederer and colleagues demonstrated increased MD in the genu of the CC and right cingulum in chronic MA users (Lederer et al. 2015). DTI, combined with cognitive measures, could potentially provide a useful marker of MA induced alterations in human behaviour and response, for example, predicting treatment outcomes in a rehabilitation setting.

Our hypothesis was that WM abnormalities would follow a predominantly fronto-subcortical pattern in MA dependent individuals and would be associated with neurocognitive dysfunction represented by WM tracts in these areas. We predicted that group differences would be present in the following major frontal WM pathways: genu and body of the corpus callosum (CC) and right and left cingulum bundle (CB); right and left uncinate fasiculus (UF); the superior longitudinal fasciculus (SLF) and the anterior corona radiata (CR). The selection of the aforementioned WM tracts is based on the ICBM-DTI-81 white-matter atlas and represents tracts previously reported on in a separate cohort of methamphetamine patients by our lab (Mori et al. 2008; Uhlmann et al. 2016).

This study was approved by the University of Cape Town Human Research Ethics Committee (HREC no: 635/2014), and was conducted in accordance with the principles laid down by the Declaration of Helsinki 2013.

2. Methods and materials

2.1. Subjects

All MA dependent individuals, aged 18-40 years, attending the Tafelsig Matrix Rehabilitation Centre in Cape Town, South Africa, during the period of December 2011 to December 2012 were invited to participate in the study. Healthy, non-using, control subjects of a similar age were recruited in a convenience fashion from friends and family of MA + users living within the same community. Inclusion criteria for MA + participants were as follows: (1) completed 7 years of formal schooling; (2) right-handed; (3) able to converse

in English or Afrikaans and (5) MA dependence, as confirmed by the Mini International Neuropsychiatric Interview (MINI-500)(Sheehan et al. 1997).

Participants were excluded from the study if they were found to have the following: (1) a history of past or current serious mental illness (e.g. schizophrenia, bipolar mood disorder); (2) a history of past or current neurological illness affecting the brain (e.g. cerebrovascular illness, epilepsy); (3) severe systemic illness or significant head injury (loss of consciousness > 30mins and/or requiring an admission to hospital); (4) past or current substance abuse other than MA; alcohol; cannabis or nicotine; (5) HIV seropositivity; (6) contraindication to MRI and (7) refusal to sign informed consent. MA+ participants were required to abstain from MA use for 3 days prior to the neuropsychological assessment and 7 days prior to the neuroimaging. This was confirmed on self-report and by means of a urine drug screen (UDS) for amphetamines; opiates; cannabis and benzodiazepines. The Drug Use Disorders Identification Test (DUDIT), a sensitive, 11-item self-report tool, was used to screen for any substance use, in addition to methamphetamine (A. H. Berman et al. 2005). Healthy controls were excluded for any substance use, including cannabis. The DUDIT was used in conjunction with the Alcohol Use Disorders Identification Test (AUDIT), a widely used, validated 10-item tool allowing investigators to screen for excessive alcohol use (Bohn et al. 1995).

Measurement of total MA dose is notoriously difficult. In South Africa, MA is distributed in the form of straws at a cost of 40-60 ZAR per straw (the length of a drinking straw is filled with MA and divided into segments). Ten straws are estimated to hold an equivalent of 1 gram of MA, although this measurement may be inaccurate as the MA is often combined with other substances, such as talcum powder. For this study participants were asked to report dose as either number of straws or grams used per day or both. All participants signed full informed consent prior to entry in the study.

2.2. Neuropsychological assessment

The cognitive evaluation occurred on a single test day and was undertaken in the participant's first language (English or Afrikaans). The neuropsychological (NP) test battery was selected by a trained local neuropsychologist (HG) with expertise in MA abuse. The domains chosen for this study represent those most frequently associated with deficits in MA abuse (Scott et al. 2007; Gonzalez et al. 2004), and include the following tests, as listed in Table 2: (1) motor speed (Grooved Pegboard Test); (2) episodic memory (Hopkins Verbal Learning Test –Revised; total and delayed); (3) attention/working memory (WAIS – III Digit Span Subtest); (4) speed of information processing (Color Trails; WAIS – III Digit Symbol Subtest; Stroop color/word); (5) verbal fluency (Letter Fluency; Category Fluency) and (6) executive function (Stroop Color and Word; Color trails -II; Hayling Test).

2.3. Diffusion tensor imaging acquisition and processing

Magnetic resonance imaging was performed on a Siemens Magnetom 3T Allegra at the Cape Universities Brain Imaging Centre (CUBIC), within one week of the neuromedical and neuropsychological assessment. DTI data was acquired with the following parameters: TR=8800ms, TE=88ms, 30 diffusion-weighted volumes with b=1000sec/mm2 and 3 unweighted volumes (b=0sec/mm2). In-plane resolution was 2×2 mm^2 with a slice thickness of 2.2 mm. The sequence was repeated 3 times to improve signal-to-noise ratio. In order to facilitate registration of the DTI data in standard space, thereby allowing group comparisons of DTI outcomes, a high resolution multi-echo MPRAGE T1-weighted image was acquired, with the following parameters: TR=2530ms; TE=1.64ms, 3.5ms, 5.36ms and 7.22ms; FOV=256x256; 176 slices, 1mm isotropic voxel size.

The 3 b=0sec/mm2 images for each DTI repetition were averaged to improve the signal-to-noise ratio. Eddy current correction was performed with the FMRIB Software Library (FSL 4.1.8) on each acquisition (Smith et al. 2006). Data were exported to Matlab (Mathworks Inc, Natick, MA) for further processing with all three acquisitions being co-registered by using the b=0 mm/s^2 as reference. The DTI acquisitions were averaged and exported to FSL for tensor fitting and brain extraction. Outliers were calculated by obtaining the

Z-value of the tensor estimates at the 25th and 75th percentile. Outliers were discarded if the data points were more than 3 standard deviations from the mean. Whole brain statistical analysis was undertaken using FSL's tract-based spatial statistics (TBSS) software program (Smith et al. 2006). The surface data produced by Freesurfer was smoothed and imported into Freesurfer's qdec toolbox for vertex-level analysis. The following ROIs were selected based on a predefined mask as per the ICBM-DTI-18 white matter-labels atlas: genu and body of the corpus callosum (CC) and bilaterally for the cingulum bundle (CB); the uncinate fasiculus (UF); the superior longitudinal fasciculus (SLF) and the corona radiata (CR)(Mori et al. 2008).

2.4. Statistical analysis

MA + users were compared with healthy controls with respect to demographic and clinical characteristics. The effect of gender on these sample characteristics was also determined. Group differences on categorical data were assessed using chi-square tests. Continuous outcomes were analysed using independent t-tests, or alternatively, non-parametric two-sided Mann Whitney or Kruskal-Wallis tests in cases in which parametric assumptions of normality and heteroscedascity were not confirmed.

Neurocognitive domains scores were calculated by normalising the raw scores from constituent tasks by means of their conversion into Z scores, and then averaging the resulting scores across the tasks within each domain. The rationale behind creating Z scores is to have standardized scores on the same scale which can be compared across domains. Z scores were obtained by substracting the mean of the scores of the healthy control group from the mean of the MA + group and dividing the result by the standard deviation of the control group. i.e. $X_{MA+} - X_{MA-}/SD\ (MA-)$. In addition, a global neurocognitive score (GNS) was derived from the mean Z score across all of the domains for each participant. Group differences in domain scores were determined using independent samples t-tests. In order to adjust for the effects of age and gender on domain scores, test estimates for between-group comparisons on the domain scores were also extracted from linear regression models with group membership, age and gender as predictor variables. In addition, non-

parametric spearman's correlations were calculated between the FA and MD measures from the tracts of interest and the GNS within each of the groups. The risk of false positives was minimised by employing bonferroni correction for multiple comparisons

All statistical analyses were conducted using the R statistical computing platform (version 3.2; R Core Team) with alpha set to 0.05 (RCoreTeam 2013).

3. Results

3.1. Demographic data

21 MA abusers (48% male, mean age = 26.7 years) currently engaged in the MATRIX rehabilitation program and 19 healthy controls (26% male, average age = 27.4 years) participated in this research study. The study groups were comparable with respect to age, education, housing and relationship status (see Table 1). There was no difference in alcohol use between the two groups as measured on the AUDIT ($p>0.1$). There was preliminary evidence that concurrent cannabis use was greater in male MA subjects, though this was not statistically significant ($p=0.06$).

3.2 MA use characteristics

The average age of first MA use was 19.6 years of age. Most MA users engaged in daily use of the drug. Males and females did not differ with respect to age of first use of MA, duration of use or abstinence, amount of money spent on MA per week, and the frequency or estimated amount of MA used (see Table 1).

3.3. Neuropsychological testing

No statistically significant differences in neurocognitive performance were found between MA dependent individuals and healthy comparison subjects for any of the cognitive domains (Table 2).

3.4. Diffusion tensor imaging analysis

3.4.1 Effect of MA on mean diffusion (MD)

Regression models of group differences for the frontal tracts of particular interest in this study (genu and body of the CC, bilateral cingulum bundle; bilateral uncinate fasciculus; bilateral anterior corona radiata; bilateral superior longitudinal fasciculus) revealed a sub-threshold increase in MD in the right uncinate fasciculus ($t = -1.79$, $p = 0.08$), after adjusting for the effects of age and gender (Table 3). Age had a significant impact on several ROIs after adjusting for gender and MA use. These, however, did not survive bonferroni correction for multiple comparisons.

Given lack of evidence of group differences in the planned comparisons, post-hoc exploratory univariate comparisons of mean diffusion for forty-eight ROIs between the groups was conducted. These additional analyses detected significantly increased MD in the external capsule only (unadjusted $p = 0.02$) in MA participants (Table S2).

3.4.2 Effect of MA on fractional anisotropy (FA)

There was no evidence from regression models for group differences between the tracts of interest (Table 3). Sub-threshold evidence of a decrease of FA in the left superior longitudinal fasiculus relative to the control group was observed in MA+ patients, however ($t = 1,96$, $p = 0,06$).

Additional post-hoc exploratory univariate DTI comparisons for the 48 ROIs revealed no significant differences in FA between MA dependent users and healthy controls (Table S1). Greater age was associated with a significant reduction in FA in the right uncinate fasiculus ($p=0,04$), after adjusting for gender and group status.

3.4.3 Relationship between neuropsychological performance and DTI in selected ROIs

Greater FA in the left uncinate fasciculus was associated with an impaired global neurocognitive score in MA dependent individuals (Rho = -0.48, p = 0.04), but not in controls (Rho = 0.05, p = 0.85; see Table 4), a difference which did not survive correction for multiple comparisons. MD scores were not informative with respect to cognitive impairment for any of the ROIs.

4. Discussion

In this pilot study of young adult, MA dependent users, we were not able to demonstrate significant differences in neuropsychological performance or white matter abnormalities on DTI when compared with healthy control participants. No significant between-group findings for both FA and MD were observed in our tracts of interest. After adjusting for age and gender, though, a sub-threshold decreased FA was observed in the left superior longitudinal fasiculus in MA+ patients, and increased MD in the right uncinate fasiculus. The above findings were consistent with our hypothesis and in keeping with reported DTI findings in the MA literature. In addition, increased FA in the left uncinate fasciculus was associated with poorer neuropsychological performance, as measured by a global neurocognitive score, however, none of these findings survived correction for multiple comparisons.

A number of factors may have contributed to the negative findings of this study. The average age of our study sample (26.7 years) was considerably younger than populations reported in the MA / DTI literature. For instance, Salo and colleagues (2009) demonstrated WM abnormalities in the CC, correlating with poor cognitive control, in MA users with an average age of 36.29 (±8.7) years. Kim et al. (2009) reported on MA dependent subjects with an average age of 34.4 (±2.9) years and showed decreased FA in the CC, which corresponded with a worse performance on the Wisconsin Card Sorting Test (WCST). Younger age may be protective in MA use, raising the possibility of an initial adaptive response to MA due to increased neuronal plasticity in younger brains, as suggested by Berman and colleagues (Berman et al. 2008). Furthermore, a temporary imbalance in the frontostriatal circuits due to differences in maturation rates between the cortex and striatum has

been suggested by Vink and colleagues (Vink et al. 2014). This may serve to explain some variability in the neuropsychological task performance. Our sample also had a relatively shorter duration of MA use in comparison to other study populations (7.4 ± 3.65 vs. 10.6 ± 2.7 years in Kim et al. (2009)), which may have impacted on the severity of neurotoxicity and subsequent WM damage. Furthermore, the effects of gender should be considered, particularly with regards to MA use characteristics. Although our sample was small and differences between male and female participants were not significant, male MA users engaged in more frequent MA use; had a longer duration of use and were more likely to abuse cannabis. A larger sample may reflect both DTI and neuropsychological differences associated with these differences.

In keeping with Chung et al. we expected to find lower FA values in the prefrontal and frontal cortices (Chung et al. 2007). Microstructural WM abnormalities are present within 7-13 days of abstinence from MA, notably in the prefrontal cortex and hippocampal formation (Tobias et al. 2010). The duration of abstinence in our sample ranged from an average of 38.8 days (range: 10-65 days) in male subjects to 60 days (range: 24-126 days) in female MA users. The absence of WM abnormalities in this sample is unexpected and may indicate the possibility of neuronal recovery during the abstinent period. Nordahl et al. (2005) suggest a period of normalization of neuronal structure and function following MA abstinence, supported by the normalization of choline levels on magnetic resonance spectroscopy in the anterior cingulum. Wang et al demonstrated recovery of thalamic metabolism in abstinent MA users and suggest that this may reflect a compensatory response to the dopaminergic deficit present in the striatum, subsequently improving neuropsychological performance (Wang 2004). Furthermore Volkow et al. (2001) demonstrated the recovery of the dopamine transporter following protracted abstinence from MA. FA is a measure of the movement of water molecules within WM tracts. FA is thought to increase when this diffusion is highly ordered within intact WM. Our finding of a sub-threshold positive correlation between increased FA in the left superior fasiculus and a measure of global cognitive performance in healthy controls supports this interpretation of FA as an index of WM integrity. The cingulum forms part of

the frontal-striatal network of WM, essential for higher-order executive functioning. Nevertheless we were unable to demonstrate a significant deficit in executive function in MA dependent individuals.

Limitations

The sample size of this study is small, though comparable to other DTI studies of MA use (Chung et al. 2007, Kim et al. 2009), and a larger sample may have provided sufficient power to yield positive findings. We were not able to include the WCST in our neuropsychological test battery, known to be associated with DTI abnormalities (Chung 2007, Kim 2009), due to time and resource constraints. MA users are expected to have comparatively lower mean scores for the neurocognitive tests, yet may still perform within the normed limits for their age and education (Hart et al. 2011). The lack of group differences in test performance may reflect a selection bias within the healthy comparison group. The healthy control group was selected predominantly from family and friends accompanying the MA dependent user to their rehab visits and alcohol use was not excluded. Female healthy controls (n = 14) were mostly unemployed and had the highest scores on the AUDIT, which may, in turn, have contributed to impaired test scores.

Due to the frequent co-morbidity of co-morbid cannabis use in this population, we elected not to exclude cannabis users for the MA+ group. Male MA users trended towards an increased co-morbid use of cannabis ($p=0.06$). Although not statistically significant, a larger sample size may have highlighted this potential difference between genders with implications for between group differences. Gonzalez et al. (2004) postulated a protective effect of cannabis on neurocognitive performance in MA abuse, yet were unable to demonstrate this. The authors concluded that comorbid cannabis use did not exacerbate the neurotoxic properties of MA, and recommended further studies of the potentially neuro-protective qualities of cannabis in the context of MA use (Gonzalez et al. 2004).

Dosage is an important aspect of understanding the neurotoxic potential of MA. We attempted to determine an estimate of the dosage (based on self-

report by MA dependent individuals), however, we feel that these estimates are highly inaccurate as MA in South Africa is frequently cut and sold with other inert substances and is therefore difficult to measure. Although the authors recommend a more accurate measurement of MA potency in future studies, we are aware that this is a near impossible task due to the variability of substance produced in informal MA labs.

Conclusion

The absence of neuropsychological or white matter deficits in this relatively young adults group of MA dependent individuals may suggest a neuroprotective aspect associated with younger age. There may indeed be a tipping-point, whereby neurotoxicity is reflected on cognitive measures and neuroimaging. The authors recommend further research examining the effects of age; co-morbid substance abuse and HIV co-infection using a matched-control group.

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Declaration of conflicts of interest

The authors report no conflicts of interest.

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Table 1: Participant demographic data and clinical characteristics

Description	MA+ (n=21)		MA - (n=19)		Test statistics[a]
	Male (n=10)	Female (n=11)	Male (n=5)	Female (n=14)	
Age, years, mean (SD)	28.55 (5.43)	25.05 (4.70)	29.59 (5.97)	26.66 (5.32)	t = 2.91,(3), p = 0.41
Years of formal education, mean (SD)	10.2 (2.49)	10.27 (2.2)	10.2 (2.49)	10.21 (1.31)	t = 0.23,(3), p = 0.97
Unemployed, %	10 (100%)	9 (82%)	2 (40%)	10 (71%)	χ^2= 8.89, (4), p = 0.06
Single, %	9 (90%)	9 (82%)	3 (60%)	7 (50%)	χ^2= 8.27, (4), p = 0.08
Living in family home, %	9 (90%)	9 (82%)	2(40%)	8 (57%)	χ^2= 7.77, (4), p = 0.10
Audit mean, (SD)	1.7, (2.1)	0.8, (1.7)	0.6, (1.3)	2.4,(3.1)	t = 3.18, (3), p = 0.36
Dudit mean, (SD)	21.4, (8.9)	19.3, (8.6)	0 (0)	0 (0)	z =-1.10, (1), p = 0.27
MA use characteristics					
Age, in years, of first use, mean, (SD)	20, (5.54)	19.11, (4.83)	-	-	Z=-1.10, p = 0.71
Weekly cost of MA, %: <R500 R500 to R1000 R1000 to R2000 >R2000	 5 (50%) 2 (20%) 2 (20%) 1 (10%)	 8 (89%) 1 (11%) - -	-	-	χ^2= 3.98, (3), p = 0.26
Frequency of use Daily, % Weekly, %	 9 (90%) 1 (10%)	 6 (67%) 3 (33%)	-	-	χ^2= 1.55, (1), p = 0.21
Daily dose in grams, mean, (SD)	2.17, (2.11)	1.51, (1.83)	-	-	Z = -1.07, p = 0.28
Duration of use, yrs, mean, (SD)	8.01, (4.13)	6.72, (3.12)	-	-	Z = -0.53, p = 0.59
Days abstinent, mean, (SD)	38.8, (23.0)	60.9, (38.9)	-	-	Z = 1.09, p = 0.27
Co-morbid cannabis use, %	80	40	-	-	χ^2= 3.33, (1), p = 0.06

Note: MA = methamphetamine; SD = standard deviation; AUDIT = Alcohol Use Disorders Identification Test; DUDIT = Drug Use Disorders Identification Test; IQR = interquartile range
[a] degrees of freedom in parentheses

Table 2: Neuropsychological performance of MA users and healthy comparison subjects

Variable [a]	MA + (*n* = 21)	MA – (*n* = 19)	Statistic	Adjusted statistic [b]
Motor speed				
GP Dominant	68.1 (7.87)	69.32 (8.49)		
GP Non-dominant	71.86 (9.06)	71.26 (8.69)		
Motor speed Z	0.02 (0.89)	-0.02 (0.87)	t = -0.15 (37.78), p = 0.88	t = 1.30, p = 0.20
Episodic memory				
HVLT-R total	20.86 (4.08)	20.89 (4.25)		
HVLT-R delay	7.29 (2)	6.47 (2.55)		
Episodic memory Z	0.08 (0.87)	-0.09 (1.01)	t = -0.58 (35.75), p = 0.57	t = 0.64, p = 0.53
Attention/working memory				
Digit span	11.29 (3.3)	12.11 (2.81)		
Working memory Z	-0.13 (1.0)	0.14 (0.92)	t = 0.85 (37.9), p = .40	t = -1.01, p = 0.32
Speed of information processing				
Colour trails I	51 (16.64)	43.26 (9.8)		
Digit symbol	45.48 (11.49)	50.58 (12.78)		
Stroop Color Naming	45.71 (14.54)	38.16 (11.62)		
Info speed Z	-0.12 (0.85)	0.14 0.49	t = 1.22 (32.51), p = 0.23	t = -1.07. p = 0.29
Verbal fluency				
COWAT – FAS	0.17 (0.99)	-0.19 (0.79)		
Animal fluency	33.76 (7.83)	32.37 (7.15)		
Fluency Z	0.17 (0.99)	-0.19 (0.79)	t = -1.25 (37.44), p = 0.22	t = 1.24, p = 0.22
Executive function [c]				
Stroop Color and Word Test	45.71 (14.54)	38.16 (11.62)		
Colour trails 2	92.76 (26.05)	88.11 (18.61)		
Hayling Test	6.4 (3.68)	7.47 (4.59)		
Executive function Z	0.03 (0.77)	-0.02 (0.68)	t = -0.19 (36.80), p = 0.85	t = 0.13, p = 0.90

Note: [a]All scores represent raw scores, presented as means and standard deviations. Higher domain Z scores reflect better test performance. [b] Group comparisons after adjusting for the effects of gender and age within a linear regression model [c] Data was missing for one MA + participant.
MA + = Methamphetamine user; MA - = healthy comparison subject
HVLT-R = Hopkins Verbal Learning Test – Revised; COWAT – Controlled Oral Word Association Test; GP – Grooved Pegboard

Table 3: Differences between MA+ and MA- groups on DTI outcomes for tracts of interest

Region of interest	MA+ (M +-SD)	MA- (M +-SD)	Model est. +-SD	T-test statistic	alpha
FA					
Genu CC	0.39 ±0.04	0.40 ±0.03	0,014 +-0,012	t = 1,21	p = 0,23
Body CC	0.39 ±0.04	0.39 ±0.02	0,006 +-0,011	t = 0,48	p = 0,63
R Cingulum	0.38 ±0.04	0.40 ±0.03	0,020 +-0,012	t = 1,61	p = 0,12
L Cingulum	0.41 ±0.03	0.41 ±0.02	0,004 +-0,009	t = 0,49	p = 0,63
R Uncinate fasciculus	0.32 ±0.05	0.33 ±0.04	0,006 +-0,015	t = 0,37	p = 0,71
L Uncinate fasciculus	0.45 ±0.04	0.45 ±0.02	0,007 +-0,010	t = 0,66	p = 0,51
R Ant corona radiata	0.49 ±0.04	0.49 ±0.03	0,009 +-0,012	t = 0,78	p = 0,44
L Ant corona radiata	0.48 ±0.04	0.49 ±0.02	0,005 +-0,010	t = 0,53	p = 0,60
R Sup long fasciculus	0.38 ±0.04	0.37 ±0.04	-0,009+-0,013	t = -0,73	p = 0,47
L Sup long fasciculus	0.34 ±0.04	0.36 ±0.02	0,019 +-0,010	t = 1,96	p = 0,06*
MD					
Genu CC	0.75 ±0.03	0.75 ±0.03	0,051 +-0,103	t = 0,49	p = 0,62
Body CC	0.72 ±0.04	0.73 ±0.02	0,056 +-0,0965	t = 0,58	p = 0,56
R Cingulum	0.82 ±0.03	0.82 ±0.03	-0,048 +-0,091	t = -0,53	p = 0,60
L Cingulum	0.70 ±0.03	0.70 ±0.03	-0,057 +-0,082	t = -0,69	p = 0,49
R Uncinate fasciculus	1.38 ±0.15	1.31 ±0.13	-0,759 +-0,425	t = -1,79	p = 0,08*
L Uncinate fasciculus	0.68 ±0.03	0.68 ±0.03	-0,027 +-0,105	t = -0,26	p = 0,79
R Ant corona radiata	0.93 ±0.08	0.94 ±0.06	0,031 +-0,238	t = 0,13	p = 0,89
L Ant corona radiata	0.83 ±0.06	0.82 ±0.04	-0,058 +-0,157	t = -0,37	p = 0,71
R Sup long fasciculus	0.81 ±0.08	0.83 ±0.09	0,368 +-0,268	t = 1,37	p = 0,18
L Sup long fasciculus	1.48 ±0.14	1.43 ±0.13	-0,630 +-0,435	t = -1,45	p = 0,16

Model coefficients extracted from multiple linear regression of DTI outcomes on group membership, adjusting for differences in gender and age.
MD values multiplied by 1000
*p<0.1, **p<0.05

Table 4: DTI correlates of Global Neurocognitive Score

Region of interest	rho MA+	alpha	rho MA-	alpha
FA				
Genu CC	-0.356	p = 0.12	-0.03	p = 0.92
Body CC	-0.30	p = 0.19	0.04	P = 0.88
R Cingulum	-0.14	p = 0.56	0.31	p = 0.20
L Cingulum	-0.26	p = 0.28	-0.08	p = 0.75
R Uncinate fasciculus	-0.17	p = 0.48	-0.14	p = 0.58
L Uncinate fasciculus	-0.48	p = 0.04	0.05	p = 0.85
R Anterior Corona Radiata	-0.15	p = 0.52	0.36	p = 0.13
L Anterior Corona Radiata	-0.24	p = 0.31	-0.04	p = 0.87
R Superior longitudinal fasciculus	-0.23	p = 0.33	0.20	p = 0.42
L Superior longitudinal fasciculus	-0.29	p = 0.21	0.05	p = 0.85
MD				
Genu CC	0.32	p = 0.17	0.05	p = 0.83
Body CC	0.29	p = 0.22	-0.12	p = 0.62
R Cingulum	0.21	p = 0.38	-0.03	p = 0.91
L Cingulum	0	p = 1	-0.07	p = 0.79
R Uncinate fasciculus	0.09	p = 0.72	0.36	p = 0.13
L Uncinate fasciculus	-0.7	p = 0.78	0.03	p = 0.90
R Anterior Corona Radiata	0.38	p = 0.10	-0.11	p = 0.66
L Anterior Corona Radiata	0.26	p = 0.26	0.07	p = 0.78
R Superior longitudinal fasciculus	0.14	p = 0.55	-0.45	p = 0.05
L Superior longitudinal fasciculus	0.22	p = 0.35	0.01	p = 0.97

MA+ = MA dependent
Bonferroni corrected p = 0.00

Supplementary material

Table S1: Mean DTI values in MA dependent individuals and healthy control subjects

Region	FA Ma +	FA MA-	FA: t value, *p value*	MD* MA+	MD* MA-	MD: t value, *p value*
Middle cerebellar peduncle	0.43 ±0.04	0.43 ±0.02	t = -0.75, p = 0.46	0.71 ±0.03	0.71 ±0.03	t = -0.08, p = 0.93
Pontine crossing tract	0.47 ±0.04	0.47 ±0.02	t = -0.64, p = 0.54	0.69 ±0.02	0.68 ±0.02	t = 1.08, p = 0.29
Genu CC	0.39 ±0.04	0.40 ±0.03	t = -1.12, p = 0.27	0.75 ±0.03	0.75 ±0.03	t = -0.23, p = 0.82
Body CC	0.39 ±0.04	0.39 ±0.02	t = -0.29, p = 0.78	0.72 ±0.04	0.73 ±0.02	t = -0.41, p = 0.68
Splenium CC	0.45 ±0.03	0.45 ±0.03	t = -0.04, p = 0.97	0.96 ±0.07	0.96 ±0.03	t = 0.08, p = 0.94
Fornix	0.43 ±0.03	0.43 ±0.02	t = -0.36, p = 0.72	1.03 ±0.08	1.03 ±0.04	t = 0.09, p = 0.93
R Corticospinal tract	0.56 ±0.04	0.56 ±0.02	t = -0.41, p = 0.69	0.75 ±0.03	0.74 ±0.02	t = 1.09, p = 0.28
L Corticospinal tract	0.55 ±0.04	0.55 ±0.02	t = -0.52, p = 0.61	0.73 ±0.02	0.72 ±0.02	t = 0.63, p = 0.53
R Medial lemniscus	0.47 ±0.04	0.47 ±0.03	t = -0.14, p = 0.89	0.66 ±0.04	0.66 ±0.03	t = 0.15, p = 0.88
L Medial lemniscus	0.46 ±0.03	0.46 ±0.02	t = -0.60, p = 0.55	0.67 ±0.03	0.68 ±0.02	t = -0.74, p = 0.47
R Inf cerebellar peduncle	0.54 ±0.03	0.54 ±0.02	t = 0.02, p = 0.99	0.69 ±0.03	0.69 ±0.02	t = 0.26, p = 0.79
L Inf cerebellar peduncle	0.36 ±0.04	0.37 ±0.02	t = -1.03, p = 0.31	0.67 ±0.03	0.67 ±0.03	t= 0.36, p = 0.72
R Sup cerebellar peduncle	0.54 ±0.03	0.54 ±0.01	t = -0.28, p = 0.78	0.70 ±0.02	0.70 ±0.02	t = -1.18, p = 0.25
L Sup cerebellar peduncle	0.49 ±0.04	0.49 ±0.03	t = 0.22, p = 0.82	0.74 ±0.03	0.74 ±0.02	t = 0.39, p = 0.71
R Cerebral peduncle	0.47 ±0.04	0.47 ±0.03	t = -0.65, p = 0.52	0.74 ±0.03	0.74 ±0.03	t = 0.06, p = 0.95
L Cerebral peduncle	0.39 ±0.03	0.39 ±0.03	t = 0.08, p = 0.94	0.72 ±0.03	0.72 ±0.03	t = -0.74, p = 0.47
R Ant limb of int capsule	0.39 ±0.03	0.39 ±0.02	t = 0.19, p = 0.84	0.72 ±0.03	0.73 ±0.03	t = -0.72, p = 0.48
L Ant limb of int capsule	0.42 ±0.03	0.43 ±0.02	t = -0.89, p = 0.38	0.69 ±0.03	0.69 ±0.02	t = 0.28, p = 0.78
R Post limb of int capsule	0.41 ±0.03	0.41 ±0.02	t = -0.25, p = 0.80	0.69 ±0.03	0.73 ±0.03	t = -1.11, p = 0.28
L Post limb of int capsule	0.42 ±0.03	0.42 ±0.03	t = -0.79, p = 0.44	0.78 ±0.04	0.78 ±0.04	t = 0.14, p = 0.89
R Retrolenticular int capsule	0.39 ±0.03	0.40 ±0.02	t = -0.72, p = 0.48	0.79 ±0.04	0.78 ±0.03	t = 0.32, p = 0.75
L Retrolenticular int capsule	0.51 ±0.04	0.51 ±0.02	t = 0.25, p = 0.81	0.81 ±0.06	0.80 ±0.04	t = 1.09, p = 0.28
R Ant corona radiata	0.49 ±0.04	0.49 ±0.03	t = -0.48, p = 0.63	0.93 ±0.08	0.94 ±0.06	t = -0.12, p = 0.90
L Ant corona radiata	0.48 ±0.04	0.49 ±0.02	t = -0.45, p = 0.65	0.83 ±0.06	0.82 ±0.04	t = 0.28, p = 0.78
R Sup corona radiata	0.46 ±0.03	0.45 ±0.03	t = 0.45, p = 0.65	0.83 ±0.04	0.82 ±0.03	t = 1.07, p = 0.29
L Sup corona radiata	0.41 ±0.03	0.41 ±0.02	t = 0.12, p = 0.90	0.81 ±0.04	0.81 ±0.04	t = 0.70, p = 0.48
R Post corona radiata	0.34 ±0.03	0.34 0.02	t = 0.01, p = 0.99	0.73 ±0.03	0.72 ±0.02	t = 0.09, p = 0.92
L Post corona radiata	0.33 ±0.03	0.33 ±0.02	t = -0.23, p = 0.82	0.75 ±0.03	0.76 ±0.02	t = -1.44, p = 0.15
R Post thalamic radiation	0.40 ±0.04	0.39 ±0.03	t = 0.57, p = 0.57	0.72 ±0.03	0.72 ±0.02	t = 0.73, p = 0.47
L Post thalamic radiation	0.42 ±0.04	0.41 ±0.03	t = 0.22, p = 0.83	0.70 ±0.03	0.70 ±0.02	t = -0.17, p = 0.87
R Sagittal stratum	0.29 ±0.05	0.29 ±0.04	t = -0.02, p = 0.98	0.81 ±0.04	0.81 ±0.05	t = -0.19, p = 0.84
L Sagittal stratum	0.28 ±0.05	0.29 ±0.03	t = -0.31, p = 0.76	0.82 ±0.06	0.82 ±0.05	t = 0.47, p = 0.64
R Ext capsule	0.38 ±0.04	0.38 ±0.04	t = -0.44, p = 0.66	0.91 ±0.03	0.89 ±0.03	t = 2.33, **p = 0.02***
L Ext capsule	0.50 ±0.07	0.53 ±0.03	t = -1.44, p = 0.16	0.88 ±0.07	0.86 ±0.03	t = 1.30, p = 0.20
R Cingulum	0.38 ±0.04	0.40 ±0.03	t = -1.49, p = 0.14	0.82 ±0.03	0.82 ±0.03	t = 0.29, p = 0.77
L Cingulum	0.41 ±0.03	0.41 ±0.02	t = -0.21, p = 0.84	0.70 ±0.03	0.70 ±0.03	t = 0.25, p = 0.80
R Cingulum hippocampus	0.38 ±0.02	0.39 ±0.02	t = -0.85, p = 0.40	0.71 ±0.02	0.72 ±0.02	t = -0.86, p = 0.39
L Cingulum hippocampus	0.40 ±0.05	0.41 ±0.03	t = -0.72, p = 0.47	0.66 ±0.03	0.65 ±0.02	t = 0.61, p = 0.54
R Fornix stria terminalis	0.38 ±0.05	0.38 ±0.03	t = -0.15, p = 0.88	0.67 ±0.04	0.68 ±0.03	t = -0.29, p = 0.78
L Fornix stria terminalis	0.42 ±0.04	0.42 ±0.03	t = 0.19, p = 0.85	0.72 ±0.06	0.70 ±0.05	t = 0.83, p = 0.41
R Sup long fasciculus	0.38 ±0.04	0.37 ±0.04	t = 0.56, p = 0.58	0.81 ±0.08	0.83 ±0.09	t = -0.91, p = 0.37
L Sup long fasciculus	0.34 ±0.04	0.36 ±0.02	t = -1.37, p = 0.18	1.48 ±0.14	1.43 ±0.13	t = 1.18, p = 0.25
R Sup fronto occ fasciculus	0.27 ±0.03	0.27 ±0.02	t = -0.69, p = 0.49	1.75 ±0.14	1.74 ±0.12	t = 0.29, p = 0.77
L Sup fronto occ fasciculus	0.63 ±0.04	0.64 ±0.02	t = -0.81, p = 0.43	0.77 ±0.03	0.77 ±0.02	t = 0.87, p = 0.39
R Uncinate fasciculus	0.32 ±0.05	0.33 ±0.04	t = -0.22, p = 0.82	1.38 ±0.15	1.31 ±0.13	t = 1.52, p = 0.14
L Uncinate fasciculus	0.45 ±0.04	0.45 ±0.02	t = -0.29, p = 0.77	0.68 ±0.03	0.68 ±0.03	t = 0.67, p = 0.49
R Tapetum	0.46 ±0.04	0.46 ±0.02	t = -0.15, p = 0.88	0.66 ±0.03	0.65 ±0.02	t = 0.59, p = 0.56
L Tapetum	0.47 ±0.04	0.48 ±0.03	t = -0.85, p = 0.39	0.70 ±0.02	0.68 ±0.03	t = 1.76, p = **0.09****

*MD value has been multiplied by 1000

*p<0.1, **p<0.05

MA + = Methamphetamine user; MA - = healthy comparison subject

FA = fractional anisotropy; MD = mean diffusion; CC = corpus callosum; WM = white matter; Sup = superior; Inf = inferior; Ant = anterior; Post = posterior; Int = internal; Ext = external; long = longitudinal; Occ = occipital

Table S2: Adjusted group differences in DTI metrics for fractional anisotropy (FA)

ROI	Group est	Group std. err	Group t value	Group p	Age est	Age std. err	Age t value	Age p	Gender est	Gender std. err	Gender t value	Gender p	Model F statistic
Middle cerebellar peduncle	0,011	0,009	t = 1,24	p = 0,22	-0,001	0,001	t = -1,26	p = 0,22	0,018	0,010	t = 1,87	**p = 0,07***	1,53
Pontine crossing tract	0,012	0,010	t = 1,17	p = 0,25	-0,001	0,001	t = -1,14	p = 0,26	0,022	0,011	t = 2,09	**p = 0,04****	1,69
Genu CC	0,014	0,012	t = 1,21	p = 0,23	0,000	0,001	t = -0,38	p = 0,70	0,007	0,013	t = 0,59	p = 0,56	0,53
Body CC	0,006	0,011	t = 0,48	p = 0,63	0,000	0,001	t = -0,06	p = 0,95	0,011	0,012	t = 0,92	p = 0,36	0,32
Splenium CC	0,002	0,009	t = 0,24	p = 0,81	0,001	0,001	t = 0,83	p = 0,41	0,011	0,010	t = 1,14	p = 0,26	0,94
Fornix	0,004	0,009	t = 0,50	p = 0,62	0,001	0,001	t = 1,10	p = 0,28	0,010	0,009	t = 1,03	p = 0,31	1,11
R Corticospinal tract	0,007	0,011	t = 0,66	p = 0,51	-0,001	0,001	t = -1,11	p = 0,27	0,011	0,012	t = 0,90	p = 0,37	0,58
L Corticospinal tract	0,010	0,010	t = 0,95	p = 0,35	-0,001	0,001	t = -1,29	p = 0,20	0,018	0,011	t = 1,60	p = 0,12	1,19
R Medial lemniscus	0,006	0,011	t = 0,51	p = 0,61	0,000	0,001	t = -0,41	p = 0,68	0,018	0,012	t = 1,53	p = 0,14	0,78
L Medial lemniscus	0,009	0,009	t = 1,04	p = 0,30	-0,001	0,001	t = -0,61	p = 0,55	0,018	0,010	t = 1,87	**p = 0,07***	1,29
R Inf cerebellar peduncle	0,002	0,008	t = 0,27	p = 0,79	-0,001	0,001	t = -0,83	p = 0,41	0,009	0,009	t = 1,02	p = 0,32	0,45
L Inf cerebellar peduncle	0,012	0,011	t = 1,05	p = 0,30	-0,001	0,001	t = -0,65	p = 0,52	0,003	0,012	t = 0,24	p = 0,81	0,46
R Sup cerebellar peduncle	0,005	0,007	t = 0,71	p = 0,48	-0,001	0,001	t = -0,80	p = 0,43	0,013	0,008	t = 1,70	p = 0,10	1,02
L Sup cerebellar peduncle	-0,002	0,012	t = -0,14	p = 0,89	-0,001	0,001	t = -1,29	p = 0,21	-0,001	0,013	t = -0,06	p = 0,95	0,64
R Cerebral peduncle	0,010	0,012	t = 0,84	p = 0,41	-0,001	0,001	t = -0,48	p = 0,63	0,010	0,012	t = 0,81	p = 0,42	0,38
L Cerebral peduncle	0,005	0,010	t = 0,49	p = 0,63	-0,001	0,001	t = -1,25	p = 0,22	0,022	0,010	t = 2,10	**p = 0,04****	1,62
R Ant limb of int capsule	0,003	0,009	t = 0,36	p = 0,72	-0,002	0,001	t = -2,11	**p = 0,04****	0,017	0,009	t = 1,83	**p = 0,08***	2,03
L Ant limb of int capsule	0,009	0,008	t = 1,11	p = 0,27	-0,001	0,001	t = -1,04	p = 0,30	0,007	0,009	t = 0,87	p = 0,39	0,73
R Post limb of int capsule	0,005	0,008	t = 0,68	p = 0,50	-0,001	0,001	t = -1,53	p = 0,13	0,012	0,008	t = 1,47	p = 0,15	1,18
L Post limb of int capsule	0,011	0,010	t = 1,18	p = 0,25	-0,002	0,001	t = -1,76	**p = 0,09***	0,013	0,010	t = 1,26	p = 0,22	1,44
R Retrolenticular int capsule	0,010	0,009	t = 1,09	p = 0,28	-0,002	0,001	t = -2,14	**p = 0,04****	0,009	0,009	t = 0,99	p = 0,33	1,75
L Retrolenticular int capsule	-0,002	0,011	t = -0,22	p = 0,82	-0,001	0,001	t = -0,74	p = 0,47	-0,002	0,012	t = -0,20	p = 0,84	0,26
R Ant corona radiata	0,009	0,012	t = 0,78	p = 0,44	-0,001	0,001	t = -1,05	p = 0,30	0,013	0,012	t = 1,09	p = 0,28	0,66
L Ant corona radiata	0,005	0,010	t = 0,53	p = 0,60	-0,001	0,001	t = -1,35	p = 0,19	0,000	0,011	t = 0,05	p = 0,96	0,71
R Sup corona radiata	-0,003	0,011	t = -0,31	p = 0,76	-0,001	0,001	t = -0,89	p = 0,38	0,003	0,011	t = 0,26	p = 0,79	0,33
L Sup corona radiata	0,002	0,009	t = 0,21	p = 0,84	-0,001	0,001	t = -0,82	p = 0,42	0,011	0,009	t = 1,17	p = 0,25	0,54
R Post corona radiata	0,004	0,007	t = 0,56	p = 0,58	-0,001	0,001	t = -1,23	p = 0,23	0,016	0,008	t = 2,13	**p = 0,04****	1,64
L Post corona radiata	0,004	0,008	t = 0,59	p = 0,56	-0,001	0,001	t = -0,76	p = 0,46	0,011	0,008	t = 1,41	p = 0,17	0,72
R Post thalamic radiation	-0,002	0,011	t = -0,17	p = 0,87	0,000	0,001	t = 0,12	p = 0,90	0,020	0,012	t = 1,72	**p = 0,09***	1,24
L Post thalamic radiation	0,002	0,011	t = 0,19	p = 0,85	0,000	0,001	t = -0,07	p = 0,94	0,021	0,012	t = 1,74	**p = 0,09***	1,09
R Sagittal stratum	-0,002	0,016	t = -0,16	p = 0,88	0,000	0,002	t = 0,27	p = 0,79	-0,012	0,017	t = -0,69	p = 0,49	0,16
L Sagittal stratum	0,008	0,015	t = 0,54	p = 0,59	-0,001	0,001	t = -0,65	p = 0,52	0,014	0,016	t = 0,91	p = 0,37	0,36
R Ext capsule	0,005	0,013	t = 0,38	p = 0,70	-0,001	0,001	t = -0,72	p = 0,48	-0,005	0,014	t = -0,35	p = 0,73	0,35
L Ext capsule	0,028	0,017	t = 1,69	p = 0,10	-0,002	0,002	t = -1,25	p = 0,22	0,020	0,018	t = 1,10	p = 0,28	1,37
R Cingulum	0,020	0,012	t = 1,61	p = 0,12	-0,001	0,001	t = -0,48	p = 0,63	0,011	0,013	t = 0,80	p = 0,43	0,93
L Cingulum	0,004	0,009	t = 0,49	p = 0,63	-0,001	0,001	t = -0,83	p = 0,41	0,010	0,009	t = 1,02	p = 0,31	0,47
R Cingulum hippocampus	0,008	0,007	t = 1,09	p = 0,28	0,000	0,001	t = -0,68	p = 0,50	0,008	0,007	t = 1,01	p = 0,32	0,63
L Cingulum hippocampus	0,014	0,013	t = 1,10	p = 0,28	-0,001	0,001	t = -0,82	p = 0,42	0,022	0,014	t = 1,56	p = 0,13	1,03
R Fornix stria terminalis	0,006	0,013	t = 0,45	p = 0,65	-0,001	0,001	t = -0,57	p = 0,57	0,016	0,014	t = 1,17	p = 0,25	0,48
L Fornix stria terminalis	0,000	0,011	t = 0,02	p = 0,98	0,001	0,001	t = 0,52	p = 0,60	0,012	0,012	t = 1,05	p = 0,30	0,63
R Sup long fasciculus	-0,009	0,013	t = -0,73	p = 0,47	0,000	0,001	t = -0,24	p = 0,81	-0,013	0,014	t = -0,94	p = 0,36	0,49
L Sup long fasciculus	0,019	0,010	t = 1,96	**p = 0,06***	-0,002	0,001	t = -1,94	**p = 0,06***	0,021	0,010	t = 2,07	**p = 0,05***	2,72
R Sup fronto occ fasciculus	0,007	0,007	t = 0,97	p = 0,34	0,000	0,001	t = -0,41	p = 0,68	0,010	0,008	t = 1,25	p = 0,22	0,68
L Sup fronto occ fasciculus	0,011	0,011	t = 1,08	p = 0,29	-0,001	0,001	t = -1,10	p = 0,28	0,012	0,011	t = 1,09	p = 0,29	0,82

R Uncinate fasciculus	0,006	0,015	t = 0,37	p = 0,71	-0,003	0,001	t = -2,10	**p = 0,04****	0,001	0,016	t = 0,03	p = 0,97	1,61
L Uncinate fasciculus	0,007	0,010	t = 0,66	p = 0,51	-0,001	0,001	t = -1,01	p = 0,32	0,015	0,011	t = 1,38	p = 0,18	0,79
R Tapetum	0,006	0,011	t = 0,54	p = 0,59	-0,001	0,001	t = -0,54	p = 0,59	0,018	0,011	t = 1,55	p = 0,13	0,82
L Tapetum	0,013	0,011	t = 1,26	p = 0,22	-0,001	0,001	t = -0,68	p = 0,50	0,020	0,011	t = 1,70	p = 0,10	1,22

***p<0.1, **p<0.05**

MA + = Methamphetamine user; MA - = healthy comparison subject; ROI = region of interest

FA = fractional anisotropy; MD = mean diffusion; CC = corpus callosum; WM = white matter; Sup = superior; Inf = inferior; Ant = anterior; Post = posterior; Int = internal; Ext = external; long = longitudinal; Occ = occipital

Table S3: Adjusted group differences in DTI metrics for mean diffusion (MD)

ROI	Group est * 10000	Group std err * 10000	Group t value	Group p	Age est * 10000	Age std err * 10000	Age t value	Age p	Gender est * 10000	Gender std err * 10000	Gender t value	Gender p	Model F.statistic
Middle cerebellar peduncle	0,0206	0,0994	t = 0,21	p = 0,84	-0,0107	0,0096	t = -1,12	p = 0,27	0,0232	0,1060	t = 0,22	p = 0,83	0,42
Pontine crossing tract	-0,0642	0,0625	t = -1,03	p = 0,31	-0,0064	0,0060	t = -1,06	p = 0,29	-0,0202	0,0668	t = -0,30	0,76	0,89
Genu CC	0,0507	0,1030	t = 0,49	p = 0,62	-0,0224	0,0099	t = -2,25	p = **0,03****	0,0535	0,1100	t = 0,49	0,63	1,72
Body CC	0,0556	0,0955	t = 0,58	p = 0,56	-0,0214	0,0092	t = -2,31	p = **0,03****	0,0048	0,1020	t = 0,05	0,96	1,99
Splenium CC	0,0246	0,1730	t = 0,14	p = 0,89	-0,0258	0,0167	t = -1,55	p = 0,13	0,0857	0,1850	t = 0,46	0,65	0,79
Fornix	0,0517	0,2070	t = 0,25	p = 0,80	-0,0342	0,0200	t = -1,71	p = **0,09***	0,2090	0,2220	t = 0,94	0,35	1,04
R Corticospinal tract	-0,0847	0,0849	t = -0,99	p = 0,33	-0,0050	0,0082	t = -0,61	p = 0,55	-0,0062	0,0908	t = -0,07	0,95	0,52
L Corticospinal tract	-0,0636	0,0734	t = -0,87	p = 0,39	-0,0044	0,0071	t = -0,61	p = 0,54	-0,1030	0,0785	t = -1,31	0,19	1,07
R Medial lemniscus	-0,0829	0,0995	t = -0,83	p = 0,41	0,0022	0,0096	t = 0,23	p = 0,82	-0,3110	0,1060	t = -2,92	**0,01****	2,99
L Medial lemniscus	0,0477	0,0847	t = 0,56	p = 0,58	-0,0069	0,0082	t = -0,84	p = 0,40	-0,0810	0,0906	t = -0,89	0,38	0,89
R Inf cerebellar peduncle	-0,0408	0,0861	t = -0,47	p = 0,64	0,0085	0,0083	t = 1,01	p = 0,32	-0,0636	0,0921	t = -0,69	0,49	0,42
L Inf cerebellar peduncle	-0,0268	0,0894	t = -0,30	p = 0,77	-0,0095	0,0086	t = -1,09	p = 0,28	-0,0127	0,0956	t = -0,13	0,89	0,52
R Sup cerebellar peduncle	0,0221	0,0445	t = 0,49	p = 0,62	-0,0010	0,0043	t = -0,23	p = 0,82	-0,1790	0,0476	t = -3,77	**0,001****	6,03
L Sup cerebellar peduncle	-0,0621	0,0888	t = -0,69	p = 0,49	0,0192	0,0086	t = 2,24	p = **0,03****	-0,0710	0,0949	t = -0,75	0,46	1,72
R Cerebral peduncle	-0,0424	0,0967	t = -0,44	p = 0,66	0,0039	0,0094	t = 0,42	p = 0,68	-0,1600	0,1030	t = -1,54	0,13	0,79
L Cerebral peduncle	-0,0013	0,0928	t = -0,01	p = 0,99	0,0117	0,0089	t = 1,30	p = 0,20	-0,3060	0,0992	t = -3,08	**0,003****	3,44
R Ant limb of int capsule	0,0349	0,0919	t = 0,38	p = 0,71	0,0085	0,0089	t = 0,96	p = 0,34	-0,1050	0,0983	t = -1,06	0,29	0,70
L Ant limb of int capsule	-0,0709	0,0873	t = -0,81	p = 0,42	0,0243	0,0084	t = 2,88	p = **0,01****	-0,1340	0,0934	t = -1,43	0,16	2,92
R Post limb of int capsule	0,0285	0,0658	t = 0,43	p = 0,67	0,0193	0,0064	t = 3,03	p = **0,004****	-0,1820	0,0703	t = -2,59	**0,01****	4,66
L Post limb of int capsule	-0,0662	0,1110	t = -0,59	p = 0,56	0,0338	0,0108	t = 3,14	p = **0,003****	-0,1190	0,1190	t = -0,99	0,33	3,29
R Retrolenticular int capsule	-0,0556	0,1140	t = -0,49	p = 0,63	0,0253	0,0110	t = 2,30	p = **0,02****	-0,0062	0,1220	t = -0,05	0,96	1,95
L Retrolenticular int capsule	-0,1610	0,1500	t = -1,08	p = 0,29	0,0285	0,0145	t = 1,97	p = **0,06***	0,1040	0,1610	t = 0,65	0,52	2,26
R Ant corona radiata	0,0306	0,2380	t = 0,13	p = 0,89	0,0147	0,0230	t = 0,64	p = 0,53	0,0627	0,2540	t = 0,25	0,81	0,21
L Ant corona radiata	-0,0584	0,1570	t = -0,37	p = 0,71	0,0313	0,0152	t = 2,06	p = **0,05***	0,0300	0,1680	t = 0,18	0,86	1,67
R Sup corona radiata	-0,1630	0,1120	t = -1,46	p = 0,15	0,0198	0,0108	t = 1,83	p = **0,08***	-0,1490	0,1200	t = -1,25	0,22	1,69
L Sup corona radiata	-0,1170	0,1180	t = -0,99	p = 0,32	0,0148	0,0114	t = 1,30	p = 0,20	-0,1260	0,1260	t = -0,10	0,32	0,87
R Post corona radiata	-0,0610	0,0853	t = -0,71	p = 0,48	0,0131	0,0083	t = 1,59	p = 0,12	-0,2040	0,0912	t = -2,23	**0,03****	1,98
L Post corona radiata	0,0885	0,0835	t = 1,06	p = 0,29	0,0064	0,0081	t = 0,79	p = 0,43	-0,0988	0,0893	t = -1,11	0,28	1,15
R Post thalamic radiation	-0,0933	0,0880	t = -1,06	p = 0,29	0,0080	0,0085	t = 0,94	p = 0,36	-0,1260	0,0941	t = -1,34	0,19	0,88
L Post thalamic radiation	-0,0061	0,0771	t = -0,08	p = 0,94	0,0080	0,0075	t = 1,07	p = 0,29	-0,0586	0,0824	t = -0,71	0,48	0,45
R Sagittal stratum	0,0927	0,1430	t = 0,65	p = 0,52	-0,0093	0,0138	t = -0,67	p = 0,51	0,2720	0,1530	t = 1,78	**0,08***	1,07
L Sagittal stratum	-0,0733	0,1760	t = -0,42	p = 0,68	-0,0010	0,0171	t = -0,05	p = 0,96	0,0151	0,1890	t = 0,08	0,94	0,07
R Ext capsule	-0,2620	0,1120	t = -2,33	p = **0,03****	0,0116	0,0108	t = 1,07	p = 0,29	-0,0192	0,1200	t = -0,16	0,87	2,15
L Ext capsule	-0,2870	0,1760	t = -1,63	p = 0,11	0,0401	0,0170	t = 2,35	p = **0,02****	-0,1560	0,1890	t = -0,83	0,41	2,43
R Cingulum	-0,0478	0,0906	t = -0,53	p = 0,60	0,0067	0,0088	t = 0,76	p = 0,45	-0,0815	0,0969	t = -0,84	0,41	0,36
L Cingulum	-0,0571	0,0817	t = -0,69	p = 0,49	0,0200	0,0079	t = 2,53	p = **0,02****	-0,1020	0,0873	t = -1,16	0,25	2,22
R Cingulum hippocampus	0,0394	0,0663	t = 0,59	p = 0,56	0,0111	0,0064	t = 1,74	p = **0,09***	-0,0454	0,0709	t = -0,64	0,53	1,27
L Cingulum hippocampus	-0,0860	0,0984	t = -0,87	p = 0,39	0,0079	0,0095	t = 0,84	p = 0,41	-0,1100	0,1050	t = -1,04	0,30	0,58
R Fornix stria terminalis	-0,0160	0,1200	t = -0,13	p = 0,89	0,0133	0,0116	t = 1,15	p = 0,26	-0,1850	0,1280	t = -1,45	0,16	0,912
L Fornix stria terminalis	-0,2050	0,1710	t = -1,20	p = 0,24	-0,0083	0,0165	t = -0,50	p = 0,62	-0,3370	0,1830	t = -1,84	**0,07***	1,77
R Sup long fasciculus	0,3680	0,2680	t = 1,37	p = 0,18	-0,0169	0,0259	t = -0,65	p = 0,52	0,5410	0,2870	t = 1,89	**0,07***	1,47
L Sup long fasciculus	-0,6300	0,4350	t = -1,45	p = 0,16	0,0723	0,0420	t = 1,72	p = 0,09	-0,3690	0,4650	t = -0,79	0,43	1,49
R Sup fronto occ fasciculus	-0,2220	0,4400	t = -0,50	p = 0,63	0,0189	0,0425	t = 0,44	p = 0,66	-0,4010	0,4700	t = -0,85	0,39	0,28

L Sup fronto occ fasciculus	-0,0984	0,0959	t = -1,03	p = 0,31	0,0182	0,0093	t = 1,96	p = **0,06***	-0,0183	0,1030	t = -0,18	0,86	1,60
R Uncinate fasciculus	-0,7590	0,4250	t = -1,79	p = **0,08***	0,1080	0,0410	t = 2,64	p = **0,01***	-0,1060	0,4540	t = -0,23	0,82	3,29
L Uncinate fasciculus	-0,0270	0,1050	t = -0,26	p = 0,79	-0,0105	0,0101	t = -1,04	p = 0,31	0,1640	0,1120	t = 1,47	0,15	1,01
R Tapetum	-0,0222	0,0887	t = -0,25	p = 0,80	-0,0034	0,0086	t = -0,41	p = 0,69	0,1200	0,0948	t = 1,26	0,21	0,65
L Tapetum	-0,1640	0,0857	t = -1,91	p = **0,06***	0,0001	0,0083	t = 0,01	p = 0,99	-0,0886	0,0917	t = -0,97	0,34	1,36

***p<0.1, **p<0.05**

MA + = Methamphetamine user; MA - = healthy comparison subject; ROI = region of interest

APPENDICES

A. **HREC approval letter 263/2010**
B. **HREC approval letter 635/2014**
C. **MATRIX information session (slides): 28 June 2011**
D. **Candidate approval for MPhil (Neuropsychiatry) UCT**
E. **City of Cape Town permission to recruit from Matrix Clinic**
F. **Inclusion/exclusion criteria flyer for Matrix and Mitchells Plain Community Health Centre**
G. **Participant informed consent sheet**
H. **Author Guidelines**

APPENDIX A

UNIVERSITY OF CAPE TOWN

Health Sciences Faculty
Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6626 • **Facsimile** [021] 406 6411
e-mail: shuretta.thomas@uct.ac.za

06 July 2010

HREC REF: 263/2010

Dr H Gouse
GSH-HIV mental Health Group
J2, Office 76
GSH

Dear Dr Gouse

PROJECT TITLE: METHAMPHETAMINE INDUCED DEFICITS IN NEURO-AND-SOCIAL-COGNITION IN THE CONTEXT OF HIV INFECTION.

Thank you for addressing the issues raised by the Faculty of Health Sciences Human Research Ethics Committee.

It is a pleasure to inform you that the Ethics Committee has **formally approved** the above-mentioned study.

Approval is granted for one year till the 15th July 2011.

Please submit an annual progress report if the research continues beyond the expiry date. Please submit a brief summary of findings if you complete the study within the approval period so that we can close our file.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the REC. REF in all your correspondence.

Yours sincerely

Signature removed

PROFESSOR M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS
Federal Wide Assurance Number: FWA00001637.

S Thomas

APPENDIX B

UNIVERSITY OF CAPE TOWN

Faculty of Health Sciences

Human Research Ethics Committee

Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
Email: shuretta.thomas@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

29 August 2014

HREC REF: 635/2014

A/Prof John Joska
Psychiatry & Mental Health
J-Block, GSH

Dear A/Prof Joska

PROJECT TITLE: WHITE MATTER CORRELATES OF NEUROPSYCHOLOGICAL FUNCTION IN YOUNG, METHAMPHETAMINE DEPENDENT INDIVIDUALS - A DIFFUSION TENSOR IMAGING STUDY - SUB-STUDY LINKED TO 236/2010 (MPHIL CANDIDATE - DR C FREEMAN)

Thank you for submitting your sub-study to the Faculty of Health Sciences Human Research Ethics Committee for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30th August 2015.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

We acknowledge that the MPHIL student, Dr Carla Patricia Freeman will also be involved in this study.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the HREC reference no in all your correspondence.

Yours sincerely

signature removed

pp
PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN ETHICS
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

HREC 635/2014

APPENDIX C

19/05/2016

What does my brain look like?

Matrix clinic, Cape Town.
28th June 2011
Dr. Carla Freeman

Magnetic Resonance Image
MRI

How does MRI work?

- "The MAGNET"
- A powerful magnetic field is created in the bore
- Atoms are lined up
- Photographic "slices" of the brain can be seen
- Taken a step further, these images can be taken while performing a task

MRI and Safety

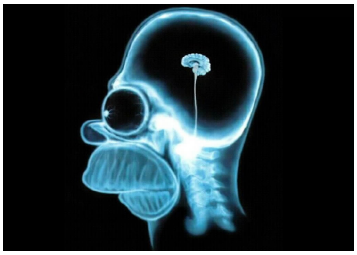
- No radiation
- Body returns to normal
- No long term damage
- Caution with metal objects

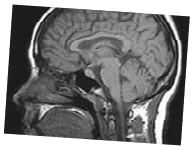
The sexiest organ in the body!

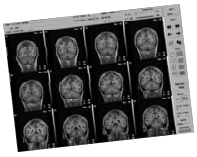
The MRI equivalent

1

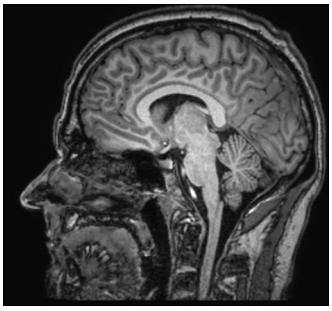
Not all brains are equal…



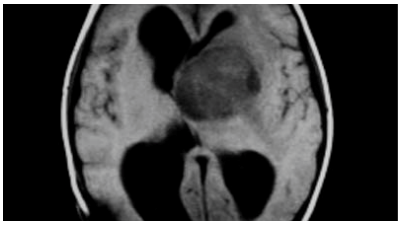




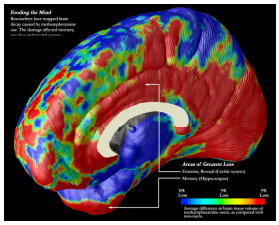
A quiz…



An example of an abnormal scan

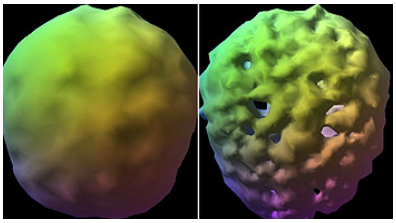


How does tik affect the brain



2

Methamphetamine and the brain…



How does tik affect the body?

"Meth mouth"

Time to reversal of the damage?

- Up to one year to reverse some effects
- The longer in a structured rehab, the better the outcome
- Longer use had worse outcomes
- RECOVERY IS DIFFICULT BUT IT IS POSSIBLE

Questions?

APPENDIX D

Re: Freeman: Confirmation of Approval of Study Proposal

John Joska <john.joska@uct.ac.za>

Mon 2015-01-26 05:04 PM

Inbox

To:Carla Freeman <FRMCAR002@myuct.ac.za>;

The whip is ever on your back...!

From: Vuyi Mgoqi <vuyi.mgoqi@uct.ac.za>
Date: Monday 26 January 2015 at 5:02 PM
To: Carla Freeman <FRMCAR002@myuct.ac.za>
Cc: Jackie Cogill <jackie.cogill@uct.ac.za>, John Joska <john.joska@uct.ac.za>
Subject: Freeman: Confirmation of Approval of Study Proposal

Dear Dr Freeman

Candidature Approval (FRMCAR002)

Degree	MPhil in Neuropsychiatry
Title	White matter correlates of neuropsychological function in young methamphetamine dependent individuals – a diffusion tensor imaging study
Department	Psychiatry and Mental Health
Supervisor	A/Prof J Joska
Ethics Approval	635/2014

I am pleased to advise that the Chair of the Dissertations/Doctoral & Masters Committee has approved your candidature for the above degree on behalf of the Committee. Formal approval was obtained by publication in the Dean's Circular, PG-Med Nov-Dec2014.

Yours sincerely

Vuyi Mgoqi

Vuyiseka Mgoqi |Receptionist: PG Academic Administration |Faculty of Health Sciences | University of Cape Town | Room N2.19, Wernher & Beit North, Health Sciences Campus, Anzio Rd, Observatory, 7925 | (+ 2721 406 67516 + 27 21 406 6584 |Office Hours: 08h30 - 16h30 Unavailable Hours: 13h00 - 13h30

APPENDIX E

Civic Centre
12 Hertzog Boulevard
Cape Town 8001
P O Box 2815, Cape Town 8000
Ask for: Dr G H Visser
Tel: 021 400-3981
Cell: 083 298 8718
Fax: 021 421-4894
E-mail: helene.visser@capetown.gov.za
Website: http://www.capetown.gov.za
Ref:
Filename: G:\Research\Gouws 10207.docx

Iziko loLuntu
12 Hertzog Boulevard
Cape Town 8001
P O Box 2815, Cape Town 8000
Cela: Qrh G H Visser
Umnxeba: 021 400-3981
Cell: 083 298 8718
Ifeksi: 021 421-4894

Burgersentrum
Hertzog-boulevard 12
Kaapstad 8001
Posbus 2815, Kaapstad 8000
Vra vir: Dr G H Visser
Tel: 021 400-3981
Sel: 083 298 8718
Faks: 021 421-4894

CITY HEALTH — Specialised Health

2010-11-22

Dear Dr Gouws

re: Research Request: Methamphetamine induced Deficits in Neuro-and-Social cognition in the Context of HIV Infection (ID: 10207)

Permission has been granted to do the research as per your protocol at the following City Health Matrix Sites (which include the required recruitment of HIV positive & control clients from the same clinics), Tafelsig, Table View and Delft South.

Your request to also recruit clients from adjoining ARV sites was not approved due to the following complexities: Tafelsig and Table View don't have ARV clinics and the nearest ARV site would be Mzamomhle and Du Noon respectively. However, these 2 sites are very busy, space constrained sites, not suitable for research recruitment.

The Health Management Team therefore recommended that you start with the Matrix Sites and approach us again at a later stage if enrolment is too slow and we could consider other sites to recruit the HIV positive clients from.

Please note the following:

1. Any individual patient information obtained must be kept confidential.
2. Access to the facilities must be arranged with the relevant Managers such that normal activities are not disrupted.
3. A copy of your final report should be sent to the City Health Head Office, P O Box 2815 Cape Town 8001, within 3 months of its completion and feedback must also be given to the clinics involved.
4. Your project has been given an ID Number (10207). Please use this in any future correspondence with us.

Thank you for your cooperation and please contact me if you require further information or assistance.

Yours sincerely

signature removed

DR G H VISSER
MANAGER: SPECIALISED HEALTH

cc. Mrs Elloker & Mrs Ngana
Mrs Sifanelo & Mrs Stanley
Mr Nkoko & Mrs Nojaholo
Dr Jennings
Mrs Bosch

APPENDIX F

1st Floor
Anzio Road
Groote Schuur Hospital
Observatory
Cape Town
7925

Tel: +27 21 404 5222
Fax: +27 21 448 8158
Email:
Dr Hetta Gouse
hetta.gouse@uct.ac.za
Dr Carla Freeman
carla.freeman@uct.ac.za
Website: www.hivmentalhealth.co.za

THIS PATIENT IS PART OF THE GSH-HIV MENTAL HEALTH GROUP RESEARCH PROJECT. AN MRI SCAN IS AVAILABLE ON REQUEST.

Inclusion criteria

a) Between 18 and 40 years old.
b) Conversational in English.
c) HIV+ HAART naïve with CD4 cell count of ≤350.
d) Literate with minimum grade 7 (Std 5) education.

Exclusion criteria

a) Past and present comorbid psychiatric disorders.
b) Past and present comorbid neurological disorders.
c) Traumatic head injury with loss of consciousness for longer than 30 minutes, AND/OR requiring overnight admission to hospital.
d) Contra-indications to MRI – such as pregnancy, metal or claustrophobia.

APPENDIX G

MATRIX PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM

Methamphetamine induced deficits in neuro- and social-cognition in the context of HIV infection.

PRINCIPAL INVESTIGATOR: Dr John Joska CONTACT NUMBER: 021-404 2154
ADDRESS: Department of Psychiatry and Mental Health, J-block, Groote Schuur Hospital, Anzio Road, Observatory, 7925

As you enter Matrix treatment some information will be collected from you by the clinic staff. You will also be required to do an HIV test. We would like to use some of this information, including your HIV status, to help us understand drug addiction better and request your permission for access to this information. After this you may be contacted to be screened to see if you meet the criteria to partake in the rest of the research study which consists of two parts, neuropsychological testing and a MRI scan. You will be interviewed in order to find out if you have any mental problems which may affect your future health or your ability to function.

During the first visit at Groote Schuur Hospital you will perform a series of tests in order to see how well you can think, remember and do certain things. It will take about 3 hours. This will be followed by a medical examination performed by a medical doctor. Voluntary pre and post-test counselling will accompany a blood test to confirm your HIV status. These results will remain strictly confidential. Two additional tubes of blood will be taken for genetic testing. This blood will NOT be used to diagnose genetic illnesses. It will only be used to further understand how drugs like methamphetamine and illnesses like HIV affect the brain. These results will be kept confidential and will not impact on your current or future care and treatment. This procedure involves one needle-prick and we will do our utmost to ensure your comfort throughout.

We would like to use this information, as well as basic information about your medical condition to study the problems that people with HIV/AIDS AND/OR tik use (methamphetamine) might have. It will be kept private and not linked to your name. If your doctor or Matrix Clinic staff requests it, a report on your condition will be given to your clinic doctor or counsellor who referred you. They will use this to make a decision on your treatment; furthermore it may lead to better treatment for others in the future.

This study may make you feel uncomfortable as you talk about mental health problems. You may feel embarrassed or shy. Sometimes painful information is shared. Also, some people feel that is it better not to know about memory or thinking problems.

During the second visit in this study you will have a type of brain scan which will be done at the Cape Universities Brain Imaging Centre at Tygerberg Hospital. This scan is called an MRI (magnetic resonance imaging) scan. The scan will require you to lie on your back on a table that will move into the scanning machine for the 60 minutes it will take for the scan. During most of this time you will be able to close your eyes and rest. You will also be able to talk to the study doctor/assistant at all times during the scan if you should experience any discomfort. You will do two tasks during which you will be asked to to push a button. The scan is a safe procedure if you have been screened

correctly for the presence of any magnetic material on or inside you such as pace-makers, surgical clips and metal objects in the eyes. A formal screen for this will be done at the screening visit by a member of the study team. As the scan is done in a relatively confined space, occasionally people become anxious. This does not happen often, and if you feel anxious, we will spend time allowing you to get used to the surroundings before we begin. When the magnet in the machine is switched on, it will make some loud banging noises, but you will be clearly warned when this will take place. At this time you will feel nothing and the noise is not harmful to you in any way. To minimise the possible discomfort associated with this, we will give you some soft earplugs and will also put earphones on so that you can listen to music if you so choose.

These are the main risks. You should feel free to mention your feelings or concerns to any member of the study team.

You should feel free not participate or to not answer any question you are uncomfortable with. We would like you to sign this page to say that you allow us to use this information, but you are free NOT to. This will not affect your care and treatment in any way. It remains between you and the interviewer.

Methamphetamine users only
The Matrix clinic staff requested that we make some of the information that we collect available to them. We are going to ask you some personal questions, including questions about your sexual behaviour over the last four months. Should you not want this or any other information to be made available to the Matrix clinic staff please indicate it by ticking the box below We would like to contact you in six months for a follow up study.

☐ DO NOT MAKE MY INFORMATION AVAILABLE TO MATRIX CLINIC STAFF.

This study has been approved by the Human Research Ethics Committee (HREC) of the Faculty of Health Sciences of the University of Cape Town and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research. **For any queries regarding your rights as a participant, please contact the investigator, Dr Hetta Gouse (021 404 5224), Dr John Joska (021 404-2154) or the HREC at 021-4066411.**

Please answer the questions as honestly as possible. Ask for help if you need it.

Patient: "I understand what this interview is about and agree that the information may be used to study mental problems in people with HIV/AIDS"

Signed at (*place*) ..... on (*date*) ……..20___.

…… ……
Signature of participant **Signature of witness**

Investigator/nurse/counsellor: "I have explained this study to the patient in a way that they understand and allowed them to ask questions
I *(name)* …… declare that:

…… ……
Signature of investigator **Signature of witness**

APPENDIX H

PSYCHIATRY RESEARCH: NEUROIMAGING

The Official Publication of the International Society for Neuroimaging in Psychiatry

AUTHOR INFORMATION PACK

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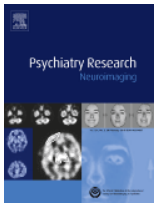

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The abstract should be followed by up to seven key words should be listed which accord with the indexing conventions of Index Medicus. Note that the keywords should not duplicate words used in the title of the article, which will be automatically indexed.

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Bernstein, T.M., 1985. The Careful Writer: A Modern Guide to English Usage. Atheneum, New York.
Buchsbaum, M.S., 1990. Frontal lobes, basal ganglia, temporal lobes--three sites for schizophrenia? Schizophrenia Bulletin 16, 377-378.
Buchsbaum, M.S., Holcomb, H.H., DeLisi, L.E., Hazlett, E., 1986. Brain imaging in affective disorders. In:Rush, A.J., Altshuler, K.Z. (Eds.), Depression: Basic Mechanisms, Diagnosis and Treatment. The Guilford Press, New York, pp. 126-142.

Issa, F., Gerhardt, G.A., Bartko, J.J., Suddath, R.L., Lynch, M. Gamache, P.H., Freedman, R., Wyatt, R.J., Kirch, D.G., 1994a. A multidimensional approach to analysis of cerebrospinal fluid biogenic amines in schizophrenia: I. Comparisons with healthy control subjects and neuroleptic-treated/ unmedicated pairs analyses. Psychiatry Research 52, 237-249.
Issa, F., Kirch, D.G., Gerhardt, G.A., Bartko, J.J., Suddath, R.L., Freedman, R., Wyatt, R.J., 1994b. A multidimensional approach to analysis of cerebrospinal fluid biogenic amines in schizophrenia: II. Correlations with psychopathology. Psychiatry Research 52, 251-258.
Strunk, W., White, E.B., 1979. The Elements of Style, 3rd ed. MacMillan, New York.

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